

APPENDIX B

PENDING CLAIMS

21. An isolated polynucleotide for enhancing protein expression, said polynucleotide comprising a nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted between positions 206 and 207 of SEQ ID NO: 1, or a fragment thereof that includes said thymidine, wherein said polynucleotide or fragment enhances protein expression when incorporated downstream of an expression regulatory promoter sequence and upstream of a protein coding sequence.

22. The isolated polynucleotide according to claim 21, wherein said nucleic acid sequence has translation promoting activity to enhance expression of a nucleic acid sequence encoding a protein sequence.

23. The isolated polynucleotide according to claim 21, wherein said nucleic acid sequence by increasing IRES activity.

24. An isolated polynucleotide that enhances protein expression when included 5' of a protein coding sequence in an expression construct by promoting mRNA translation in an IRES dependent manner, said polynucleotide comprising a nucleotide sequence of SEQ ID NO: 7.

26. An isolated polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 7 over its entire length.

28. An expression vector comprising an isolated polynucleotide according to claim 21 or claim 24.

29. A host cell transformed or transfected with the vector according to claim 28.

30. A method of expressing a protein comprising the steps of:

- (a) transforming or transfecting a host cell with an expression vector according to claim 53,
- (b) growing the host cell in a medium under conditions where the cell expresses the protein.

31. A method according to claim 30, further comprising a step of isolating the protein from the cell and/or the growth medium.

33. A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26, further comprising a detectable label.

34. A probe for screening IRES-dependent translation inhibitors, comprising the polynucleotide according to claim 26, further comprising a detectable label.

35. A composition comprising the isolated polynucleotide for enhancing protein expression according to claim 21.

36. A composition comprising the isolated polynucleotide for enhancing protein expression according to claim 24.

37. A method for determining a hypervirulent hepatitis C strain, comprising the steps of:

(a) screening a biological sample for the presence of the polynucleotide according to claim 26, and;

(b) determining presence or absence of the hypervirulent hepatitis C strain from the screening step, wherein the presence of the polynucleotide identifies the hypervirulent hepatitis C strain in the biological sample and the absence of said sequence indicates the absence of said hypervirulent hepatitis C.

38. An isolated polynucleotide according to claim 21, further comprising nucleotides 1-180 of SEQ ID NO: 1.

39. An isolated polynucleotide according to claim 21 or 38, further comprising nucleotides 342-713 of SEQ ID NO: 1.

44. An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 24, wherein the 5'-untranslated region comprises a polynucleotide sequence corresponding to at least one region selected from the group consisting of pyrimidine-rich tract, Box A, Box B, a trans factor-binding site, and a combination thereof.

45. An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 44, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion, and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or proximate sequence in at least one position corresponding to a pyrimidine-rich tract, Box A, Box B and/or trans factor-binding site contained in the 5'-untranslated region.

47. The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

48. The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of Box A, Box B, a trans-binding site, and a combination thereof.

49. The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises an AUG or ATG sequence.

50. The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a part of or an entire region of IRES of viral mRNA.

51. The isolated polynucleotide according to claim 24, wherein said nucleic acid further comprises a portion of a coding region from a viral gene adjacent to the 5'-untranslated region.

52. The isolated polynucleotide according to claim 24, wherein said nucleic acid is a cDNA sequence.

53. An expression vector according to claim 28, further comprising a protein coding sequence operably inserted downstream of the polynucleotide for enhancing protein expression.

54. An isolated polynucleotide comprising nucleotide 181-341 of SEQ ID NO: 1, wherein said polynucleotide includes a thymidine inserted between position 206 and 207 of SEQ ID NO: 1.

55. An expression vector comprising a promoter sequence, a polypeptide encoding sequence, and a nucleic acid sequence of SEQ ID NO: 7 incorporated downstream of the promoter sequence and upstream of the polypeptide encoding sequence, wherein the nucleic acid sequence of SEQ ID NO: 7 enhances expression of the polypeptide by means of increasing IRES activity.

56. The expression vector according to claim 55, wherein said gene expression vector is a vector for expression in eukaryotic cells.